Study on Isozyme Electrophoresis of *Martes zibellina* L.

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Abstract Four isozymes, such as Malate dehydrogenase (MDH), Alchol dehydrogenase (ADH), Peroxidae (POD) and Esterase (EST) in six tissues (heart, liver, kidney, muscle, eye, gonad) of *Martes zibellina* L., were analyzed by means of vertical polyacrylamide gel electrophoresis (PAGE). The results indicated that the zymograms of these four isozymes in different tissues were different from each other, i.e. there existed apparent tissue- specificity in these isozymes in *Martes zibellina* L.. Characteristic enzyme band was found both in POD zymogram and in EST zymogram. Moreover, the characteristic enzyme band in POD isozyme would be of some value to sexual identification of *Martes zibellina* L.

Key words: *Martes zibellina* L., Isozyme, Electrophoresis.

Introduction

Martes zibellina L., belonging to Carnivora order, Mustelidae family, Martes genus, is a kind of rare fur-bearing animal. Its fur is the treasure of furs or pelts of wildlife, and marten coats made with it are considered as "The Fur Champion" and are well known at home and abroad.

Martes zibellina L. is mainly distributed in Russia, Mongolia, Korea, Japan and Xinjiang Province and Northeast area of China. For a long time, due to the environmental deterioration and over hunting, the amount of the populations of wild *Martes zibellina* L. has been dropping off. For this reason, in China, *Martes zibellina* L. has been classified as one of rarely preserved wildlife animals. And researches have carried out ecology, raising, breeding, etc. Especially in recent years, more reports of studies on *Martes zibellina* L. have been published both in China and in foreign countries. However, no studies on the isozymes of *Martes zibellina* L. have been reported. In our research, four isozymes: Malate dehydrogenase (MDH), Alchol dehydrogenase (ADH), Peroxidase (POD) and Esterase (EST) in six tissues of *Martes zibellina* L. (heart, liver, kidney, muscle, eye, gonad) were analyzed by vertical polyacrylamide gel electrophoresis.

Materials and Methods

Martens used in this experiment were obtained from the field working-station of Mangui Forestory Bureau located in the north of Hulunbeier League of Mongolia in January 1997. They involved two male adults and one female adult. The fresh living tissues (hear, liver, kidney, muscle, eye, gonad) were taken out and frozen for storing.

After weighed, the materials were placed in a mortar to which a little quartz and a volume of phosphate buffer (0.2 M, pH 7.2, the volume ratio to the weight of materials was 5 m1/g) were added afterwards, then the materials was homogenized, respectively. After that, the treated preparations materials was centrifugalized at 4 000 rpm in 15 min, and the supernatant was kept and centrifugalized again at 10,000 rpm in 20 min, this time the supernatant was frozen to be stored for electrophoresis.

Vertical polyacrylamide gel electrophoresis was applied to our research. The concentration of the separate gels was 7 % and that of the concentrate gels was 2.5 %. The experimental methods and techniques for genetic study was used for reference in gel staining.

Results

Malate dehydrogenase (MDH) EC. 1.1.1.37.
The electropherogram and the electrophoretic mobility (R_f)of MDH isozymes in six tissues of *Martes zibellina* L. were exhibited in Fig. 1 and Table 1, receptively.

Table 1. The electrophoretic mobility (R_f) of MDH isozymes in six tissues of *Martes zibellina* L.

	1#(♂)						2#(♀)						3#(♂)				
	H	L	K	M	E	Y	H	L	K	M	E	X	H	L	K	M	E
MDH_1	0.47	0.45	0.45	0.45	0.46	0.46	0.45	0.46	0.45	0.45	0.46	0.45	0.46	0.46		0.45	0.46
MDH_2	0.20			0.20	0.26	0.20			0.20		0.26	0.20	0.21			0.20	0.26

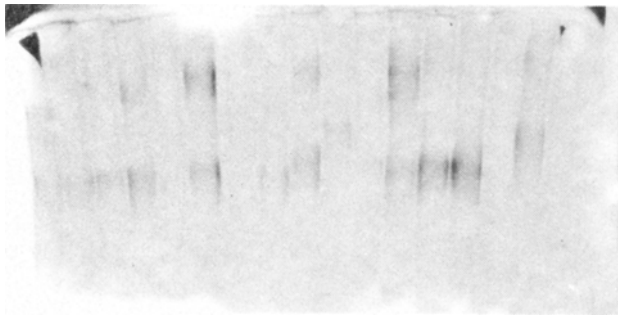

Fig. 1. **The electropherogram of MDH isozymes in six tissues of *Martes zibellina* L.**

Note: H: Heart. L: Live. K: kidney. M: Muscle. E: Eye. Y: Testis. X: Ovary

Fig. 1 and Table 1 indicated that the MDH isozyme zymograms in six tissues of *Martes zibellina* L. were apparently stored into two groups, such as s- MDH (cytoplasmic MDH isoenzymes), R_f =0.41 ~ 0. 46, and m-MDH (mitchondria MDH isozymes), R_f =0.20 ~ 0.26. Of which there was one primary band in each group, thus possessing the common characteristic of MDH isozyme. These two groups of isozymes were encoded and controlled by genes on different chromosomes. Also, there existed differences between male and female individuals and between different tissues of the same individual. There were two enzyme bamds in the heart, muscle, eye and gonad of 1# (male); in the kidney, eye and gonad of 2# (female); in the heart, muscle, eye of 3# (male); and one enzyme band in the liver, kidney of 1#; in the heart, liver, muscle of 2#; in the liver of 3#; and no enzyme band in the kidney of 3#.

Alchol dehydrogenase (ADH) EC. 1.1.1.1.

The results of electrophoresis and the electrophoretic mobility (R_f) of ADH isozymes in six tissues of *Martes zibellina* L. were showed in Fig. 2 and Table 2, respectively.

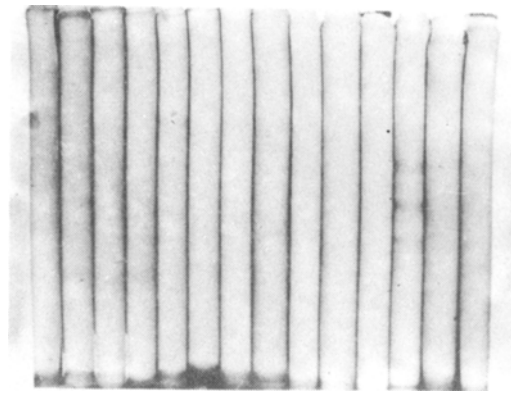

Fig. 2. The results of electrophoresis of ADH isozymes in six tissues of *Martes zibellina* L.

Fig. 2 and Table 2 denoted that the ADH isozymes in six tissues of *Martes zibellina* L. had seven phenotypes, which were encoded and controlled, by five gene loci. The liver of 1#, 2# or 3# presented four same enzyme bands; for 1# , there existed two enzyme bands in the heart, and three in the kidney, one in the eye, three in the gonad, one in the muscle. In addition, the enzyme band in the muscle of 1# was similar to that of 2#; for 2#, there was one enzyme band both in the heart and in the kidney, and two in the eye, four in the gonad.

Peroxidase (POD)

The results of electrophoresis and the electrophoretic mobility (R_f) of POD isozymes in six tissues of *Martes zibellina* L. were presented in Fig. 3 and Table 3, respectively.

Table 2. The electrophoretic mobility (Rf) of ADH isozymes in six tissues of *Martes zibellina* L.

	1#(♂)						2#(♀)						3#(♂)					
	H	L	K	M	E	Y	H	L	K	M	E	X	H	L	K	M	E	
ADH_1												0.9						*Adh*-1
ADH_2		0.68	0.72		0.67	0.67	0.67	0.70					0.69	0.69				*Adh*-2
ADH_3												0.6						
ADH_4		0.49										0.50		0.50				*Adh*-3
ADH_5	0.39	0.40	0.39			0.39			0.39	0.39	0.38	0.38						
ADH_6	0.29	0.29		0.29		0.29	0.29	0.29	0.29	0.29	0.29							*Adh*-4
ADH_7		0.22	0.22															*Adh*-5

Table 3. The electrophoretic mobility (Rf) of POD isozymes in six tissues of *Martes zibellina* L.

	1#(♂)						2#(♀)						3#(♂)					
	H	L	K	M	E	Y	H	L	K	M	E	X	H	L	K	M	E	
POD_1	0.83												0.83	0.83				*Pod*-1
POD_2	0.71												0.72					*Pod*-2
POD_3	0.45																	
POD_4	0.39			0.38	0.39	0.39	0.38			0.39	0.39	0.40				0.39		*Pod*-4

Table 4. The electrophoretic mobility (Rf) of EST isozymes in six tissues of *Martes zibellina* L.

	1#(♂)						2#(♀)						3#(♂)					
	H	L	K	M	E	Y	H	L	K	M	E	X	H	L	K	M	E	
EST_1								0.65	0.65						0.65			Est-1
EST_2								0.57					0.72					Est-2
EST_3	0.48	0.45					0.48	0.46					0.47	0.46				Est-3
EST_4		0.38																Est-4

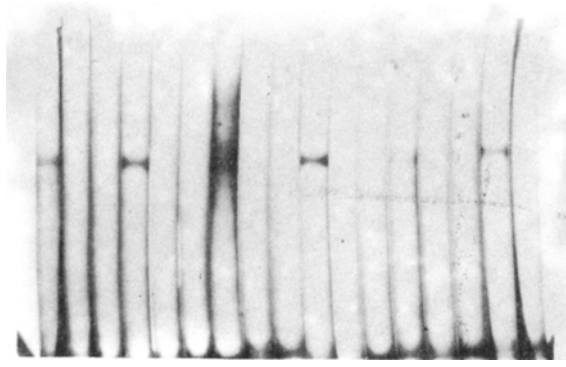

Fig. 3. The results of electrophoresis of POD isozymes in six tissues of *Martes zibellina* L.

Fig. 3 and Table 3 revealed that the POD isozymes in six tissues of *Martes zibellina* L. had four phenotypes, which were correspondent with three loci on gene (Pod -1, Pod - 2 and Pod -3). The isozyme band correspondent with the locus Pod - 3 was from the heart, muscle or gonad of individual $1^{\#}$, $2^{\#}$ or $3^{\#}$, with a R_f of 0.39. This band had a heavy staining intensity and a high enzyme content. However, for $2^{\#}$, this enzyme band assumed a dispersive band. Furthermore, another two isozyme bands correspondent with locus Pod -1 and Pod -2, existed in the heart of $1^{\#}$ as well as in the heart of $3^{\#}$ respectively, which were absent in the heart of $2^{\#}$.

Fig. 4. The results of electrophoresis of EST isozymes in six tissues of *Martes zibellina* L.

Esterase (EST)

The results of electrophoresis and the electrophoretic mobility (R_f) of EST isozymes in six tissues of *Martes zibellina* L. were showed in Fig. 4 and Table 4, respectively.

From Fig. 4 and Table 4, the results showed that the six tissues of *Martes zibellina* L., EST isozyne had four phenotypes correspondent with the loci (Est -1, Est -2, Est -3 and Est -4). Of these four phenotypes were an enzyme band with a R_f of 0.45 ~ 0.48 and with heavy band intensity and high enzyme content. This enzyme band was common to the heart and the liver of individuals of $1^{\#}$, $2^{\#}$ or $3^{\#}$. The other three enzyme bands were comparatively faint and were chiefly distributed in liver and kidney. No EST isozymes existed in muscle, eye or gonad.

Discussion

MDH isozymes were classified into two groups such as cytoplasmic isozymes (s-MDH) and mitchondria isozymes (m-MDH), genes on different chromosomes encoded them. Judging from the zymograms of the MDH isozymes in six tissues of *Martes zibellina* L., the s-MDH isozyme band that was close to the anode was common to all the tissue samples except kidney, and showed differences in enzyme content. m-MDH isozyme locus was expressed in some tissues but not in others. The zymograms of MDH isozymes demonstrated both the common characteristics of tissues and tissue specificity in *Martes zibellina* L.

The analysis of the results of electrophoresis of ADH isoenzyme brought to light that ADH isozyme had seven phenotypes by total, and each enzyme band was quite faint. Thus providing an indicator, the contents of ADH isozyme in each tissue of *Martes zibellina* L. were comparatively low. Besides, the zymograms of ADH isozyme reflected another more and slighter distinctions between tissues and between organs.

The POD isozyme of *Martes zibellina* L. had four phenotypes. Pod 4 enzyme band, common to the heart, muscle and gonad of $1^{\#}$, $2^{\#}$ or $3^{\#}$, had heavy staining intensity and high enzyme activity, and was considered as the characteristic primary band of *Martes zibellina* L.. The results showed that Pod 4 enzyme band in $2^{\#}$ (female) was dispersive. Further research was necessary to determine from what such enzyme pattern resulted. If this enzyme band was characteristic, then the zymograms of Pod 4 isozyme could be ideally applied to sexual identification of *Martes zibellina* L.

The number of the phenotypes of EST isozyme of *Martes zibellina* L. added up to four. Among them, Est

3 isozyme, common to the heart, liver of individuals of $1^{\#}$, $2^{\#}$ or $3^{\#}$, had heavy staining intensity and high enzyme content and acted as the characteristic primary enzyme hand of *Martes zibellina* L.. In addition, EST isozymes were distributed only in heart, liver and kidney, and no EST isozyme loci was expressed in muscle, eye or gonad.

To take a comprehensive review of the above analyses of the zymograms of the four isozymes (MDH, ADH, POD and EST), there existed more or less tissue-specificity as to the four isozymic systems (MDH, ADH, POD and EST) in the six tissues (liver, eye, kidney, heart, gonad, muscle, etc.) of *Martes zibellina* L.. Characteristic enzyme band was found both in the zymograms of POD isozyme and in that of EST isozyme; the characteristic enzyme band of POD isozyme was likely to avail sexual identification of *Martes zibellina* L.

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